

## Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants

K. Czerwionka<sup>1</sup>, J. Makinia<sup>1\*</sup>, K.R. Pagilla<sup>2</sup>, H.D. Stensel<sup>3</sup>

<sup>1</sup> Faculty of Civil and Environmental Engineering, Gdansk University of Technology, ul.

Narutowicza 11/12, 80-233 Gdansk, POLAND (E-mail: [kczer@pg.gda.pl](mailto:kczer@pg.gda.pl),

[jmakinia@pg.gda.pl](mailto:jmakinia@pg.gda.pl)), tel. +48 58 347-19-54, fax +48 58 347-24-21

<sup>2</sup> Department of Civil, Architectural and Environmental Engineering, Illinois Institute of

Technology, USA (E-mail: [pagilla@iit.edu](mailto:pagilla@iit.edu))

<sup>3</sup> Department of Civil and Environmental Engineering, University of Washington, Seattle,

USA (E-mail: [stensel@u.washington.edu](mailto:stensel@u.washington.edu))

### ABSTRACT

The aim of this study was to investigate the occurrence and fate of colloidal and dissolved organic nitrogen (CON and DON) across biological nutrient removal (BNR) activated sludge bioreactors.

Primary and secondary effluent total nitrogen (TN) measurements and component fractionation, CON and DON concentration profiles across BNR bioreactors, and laboratory batch experiments with

the process mixed liquor were carried out at several full-scale BNR plants in northern Poland. The

organic nitrogen (ON) components were divided into high CON, low CON, and DON based on

sequential filtration through 1.2, 0.45 and 0.1  $\mu\text{m}$  pore size filters. The average influent  $\text{DON}_{0.1\mu\text{m}}$

(<0.1  $\mu\text{m}$ ) concentrations ranged from 1.1  $\text{g N/m}^3$  to 3.9  $\text{g N/m}^3$  and accounted for only 4-13% of

total organic nitrogen. In the effluents, however, this contribution increased to 12-45% (the

$\text{DON}_{0.1\mu\text{m}}$  concentrations varied in a narrow range of 0.5-1.3  $\text{g N/m}^3$ ). Conversions of ON inside

the bioreactors were investigated in more detail in two largest plants, i.e. Gdansk (565,000 PE)

and Gdynia (516,000 PE). Inside the two studied bioreactors, the largest reductions of the

colloidal fraction were found to occur in the anaerobic and anoxic compartments, whereas an

26 increase of  $\text{DON}_{0.1\mu\text{m}}$  concentrations was observed under aerobic conditions in the last  
27 compartment. Batch experiments with the process mixed liquor confirmed that  $\text{DON}_{0.1\mu\text{m}}$  was  
28 explicitly produced in the aerobic phase and significant amounts of ON were converted in the  
29 anoxic phase of the experiments.

30

### 31 **KEYWORDS**

32 Activated sludge; biological nutrient removal; colloidal organic nitrogen; CON; dissolved organic  
33 nitrogen; DON; nitrogen fractionation; nitrogen removal

34

## 35 INTRODUCTION

36 Biological nitrogen removal (BNR) activated sludge processes are commonly used in municipal  
37 wastewater treatment plants (WWTPs) around the world to produce effluents with total nitrogen  
38 (TN) concentrations below  $10 \text{ g N/m}^3$ . Effluent TN includes total inorganic nitrogen ( $\text{NH}_4\text{-N} +$   
39  $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$ ) and total organic nitrogen (TON), which is the sum of dissolved organic  
40 nitrogen (DON), particulate organic nitrogen (PON), and colloidal organic nitrogen (CON).  
41 Biological nitrogen removal involves transformations and removal of inorganic nitrogen ( $\text{NH}_4\text{-N}$   
42 and  $\text{NO}_x\text{-N}$ ) by biomass synthesis and sludge wasting, and nitrification ( $\text{NH}_4\text{-N}$  oxidation to  $\text{NO}_2\text{-}$   
43  $\text{N}$  and  $\text{NO}_3\text{-N}$ ) and denitrification of  $\text{NO}_3\text{-N}$  and/or  $\text{NO}_2\text{-N}$  to nitrogen gas. Solid-liquid separation  
44 processes, including final clarifiers, sand filters, and membrane filters remove organic nitrogen  
45 (ON) contained in suspended solids (as PON) and removable colloidal solids (as CON). Inorganic  
46 nitrogen is of primary concern for effluent goals for TN of less than  $10 \text{ g N/m}^3$ . With stricter  
47 effluent TN permit limits becoming more common in the United States (less than  $3.0 \text{ g N/m}^3$ ),  
48 and in some cases in Europe and Japan, the contribution of effluent ON, mainly as DON and  
49 CON, has become more important and may account for 30-50% of the effluent TN (WERF,  
50 2008). Observed effluent DON contributions vary widely in municipal BNR WWTPs with  
51 reported  $\text{DON}_{0.45\mu\text{m}}$  concentrations (defined by the fraction passing through  $0.45 \mu\text{m}$  pore-size  
52 filters) ranging from <2% to as high as 85% of the effluent TN (Pagilla et al., 2006; 2008;  
53 Pehlivanoglu and Sedlak, 2004; WERF, 2008).

54  
55 Because of the importance of effluent ON in BNR WWTPs addressing low effluent TN  
56 concentration goals, understanding the fate of ON in the influent wastewater and across the  
57 activated sludge process is of great interest. Influent TN primarily consists of ammonia/ammonium  
58 ( $\text{NH}_4\text{-N}$ ) and ON plus none or little in the oxidized inorganic forms. Similar to effluent ON, the  
59 influent ON may also be characterized as the sum of PON, DON and CON. Traditionally, a  $0.45$   
60  $\mu\text{m}$  pore-size filter has been used to separate the DON (referred further to as  $\text{DON}_{0.45\mu\text{m}}$ ) from



61 PON in analytical measurements. However, in the work of Makinia et al. (2011), three different  
62 pore-size filters were used to separate the ON into PON, CON, and DON fractions. Each physical  
63 fraction was further divided into biodegradable and non-biodegradable sub-fractions. This  
64 approach resulted in accurate modeling of ON conversions in activated sludge bioreactors  
65 (Makinia et al., 2011).

66  
67 So far, very little has been done on the fate and characteristics of effluent DON in WWTPs since  
68 the early pioneering work of Parkin and McCarty (1981a,b,c), which followed DON in untreated  
69 and treated wastewater, and considered sources of DON in the activated sludge effluents,  
70 including production during biological treatment. From bench-scale tests they found the lowest  
71 effluent DON concentrations at aeration times of 6-9 hours, which corresponded to a 6-10 day  
72 aerobic solids retention time (SRT) at the studied WWTP (Palo Alto, California (USA)). Bratby  
73 et al. (2008) noted that DON concentration increases through biological treatment, and on the  
74 contrary, biological processes in activated sludge systems were identified as a potential method of  
75 DON removal (O'Shaughnessy et al., 2006; Pagilla et al., 2006). Factors influencing DON  
76 treatment efficiency include SRT, temperature, reactor hydraulics and plant perturbations. Sharp et  
77 al. (2009) found that SRT and temperature may impact both PON and DON<sub>0.45µm</sub> fraction and  
78 concentration of effluent DON for a specific plant, but were not the only factors. Studies in  
79 several WWTPs in the US and Poland revealed that the effluent concentrations of CON and DON  
80 were relatively stable regardless of the influent TN concentrations and process configurations  
81 (Pagilla et al., 2008; Sattayatewa et al., 2009b; Sattayatewa et al., 2010). Dignac et al. (2000a)  
82 found that the BNR processes can be efficient in removing low molecular weight (LMW) organic  
83 matter and DON compounds such as urea, amino acids, and proteins. In contrast, the high  
84 molecular weight (HMW) DON is considered to be inert in biological treatment (Gulyas et al.,  
85 1995; Dignac et al., 2000a; Pehlivanoglu-Mantas and Sedlak, 2008; WERF, 2008).

86



87 Present knowledge on the characteristics and behavior of CON and DON is still limited and  
88 insufficient to estimate BNR process effluent ON concentrations as a function of plant design and  
89 influent ON concentration and characteristics. The fate of ON has not been specifically studied in BNR  
90 processes and important research questions include (WERF, 2008):

91 - where DON and CON is removed or produced in BNR processes?

92 - what is the effect of BNR process design and configuration (anaerobic and anoxic contact) on  
93 effluent DON and CON?

94

95 This paper reports on the results of studies at full-scale BNR WWTPs to address these questions  
96 under the Polish conditions which are characterized by very strong municipal wastewater (e.g. TN  
97 concentrations are 2-4 times higher compared to the USA). The study evaluated influent  
98 wastewater nitrogen characteristics and the fate of nitrogen species across BNR activated sludge  
99 bioreactors at eight full-scale WWTPs in northern Poland. Batch experiments were also done at  
100 some plants to further investigate nitrogen transformations within different BNR process  
101 conditions.

102

103

## 104 **MATERIALS AND METHODS**

### 105 **Description of WWTPs**

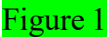

106 All of the WWTPs in the study were designed and operated for both nitrogen and phosphorus  
107 removal and varied in size and activated sludge process configurations (Table 1). The effluent TN  
108 concentration goal for the four largest facilities, with greater than 100,000 population equivalents  
109 (PE), is at the most stringent European Union (EU) standard of 10 g N/m<sup>3</sup>. The effluent TN  
110 concentration goal for the other plants is 15 g N/m<sup>3</sup>. Design configurations for enhanced  
111 biological phosphorus removal (EBPR) are used in all of the facilities except the Elblag WWTP,  
112 which has an anoxic-aerobic activated sludge process (MLE) and ferric addition in the primary



113 treatment step for phosphorus removal. The EBPR process configurations are the University of Cape  
114 Town (UCT), modified UCT (MUCT), Johannesburg (JHB) and anaerobic-anoxic-aerobic  
115 ( $A_2/O$ ). All the plants were operated over a range of SRTs due to significant seasonal activated  
116 sludge temperature fluctuations from 10-22 °C.

117  **Table 1**

118

119 In addition to the primary and secondary effluent ON fractionations, conversions of ON and  
120 organic carbon (OC) in BNR activated sludge process steps were investigated in more detail at the  
121 two largest plants, Gdansk and Gdynia WWTPs. Process schematics for these plants and the  
122 sampling locations are illustrated in  **Figure 1**. More detailed characteristics of those plants can be  
123 found elsewhere ( Makinia et al., 2006).

124  **Figure 1**

125

## 126 **WWTP measurements**

127 ***Influent-effluent analysis.*** Primary and secondary effluent 24-hour, flow-proportional composite  
128 samples were collected during ten sampling events between March, 2007 and December, 2008  
129 and analyzed for ON and OC fractions. The ON and OC fractions were based on pore-size filter  
130 separation and defined as particulate ( $>1.2 \mu\text{m}$ ), "high" colloidal ( $>0.45$  and  $<1.2 \mu\text{m}$ ), "low"  
131 colloidal ( $>0.10$  and  $<0.45 \mu\text{m}$ ), and dissolved ( $<0.1 \mu\text{m}$ ). For ON, these are defined as PON,  
132 high CON, low CON, and  $\text{DON}_{0.1\mu\text{m}}$ , respectively. In addition, between January and March, 2009,  
133 three additional measurement series were carried out with the Gdansk and Gdynia WWTPs  
134 secondary effluent 24-hour composite samples by sequential filtration through 0.1 and 0.015  $\mu\text{m}$   
135 filters to evaluate the effect of ultrafiltration on ON and OC removal.

136

137 ***ON and OC concentration profiles across BNR bioreactors.*** Conversions of non-particulate ON  
138 and OC fractions across the Gdansk and Gdynia WWTPs bioreactors were investigated by 5

139 measurement campaigns between November, 2008 and July, 2009. Average colloidal and  
140 dissolved ON and OC concentrations in the bioreactors were based on three grab samples (8 AM,  
141 11 AM and 2 PM) at the inlet and outlet from the anaerobic, anoxic and aerobic compartments.  
142 Sampling point locations at both plants are shown in **Figure 1**.

143

#### 144 **Bench-scale experiments**

145 Bench-scale experiments to evaluate the fate of ON and OC under anaerobic, anoxic, and aerobic  
146 conditions were carried out with settled wastewater and return activated sludge (RAS) seed from  
147 the Gdansk WWTP (5 tests) and Gdynia WWTP (6 tests) during the same time period as the  
148 concentration profile measurements. The experimental apparatus consisted of two parallel 4.0  
149 dm<sup>3</sup>-batch reactors with electrodes for a continuous monitoring of pH, ORP, temperature and  
150 dissolved oxygen (DO) and computer control system to maintain DO concentration and  
151 temperature around set points. This system also controlled a cyclic measurement of oxygen  
152 uptake rate (OUR) in small chambers connected to the main units. During the batch experiments,  
153 both reactors were operated in a 3-step sequence of anaerobic (2 h), anoxic (4 h, after addition of  
154 KNO<sub>3</sub>) and aerobic (6 h) conditions.

155

156 To observe the fate of only the dissolved ON and OC and the effect of PON and CON on effluent  
157 DON concentration, reactor 1 (R1) was fed settled wastewater and reactor 2 (R2) was fed pretreated  
158 settled wastewater with only DON and dissolved OC constituents (Figure 2). The rapid coagulation-  
159 flocculation method by Mamais et al. (1993) based on Zn(OH)<sub>2</sub> precipitation at pH = 10.5 was  
160 used to remove particulate and colloidal ON and OC. After removing the colloids and particulates  
161 by settling, the pH was adjusted to its original value by adding 6M HCl. The RAS seed was  
162 diluted to obtain mixed liquor suspended solids (MLSS) concentration at approx. 2.5-3 kg/m<sup>3</sup> in  
163 the reactors. The actual MLSS concentrations were measured at the beginning and end of the  
164 experiment. A heating/cooling system was set to maintain the batch reactor temperature equal to



165 the actual (current) process temperature in the full-scale bioreactors. After adding the RAS and  
166 feed wastewater to the reactors, the mixers were turned on at 180 rpm. Samples of 100-150 cm<sup>3</sup>  
167 were withdrawn at the time intervals shown in **Figure 2**, filtered under vacuum pressure on 1.2  
168 µm pore-size filter and then analyzed. The “basic” set of lab analyses comprised NH<sub>4</sub>-N, NO<sub>2</sub>-N,  
169 NO<sub>3</sub>-N, COD and PO<sub>4</sub>-P measurements, whereas the “full” set of lab analyses included additional  
170 TN and total organic carbon (TOC) measurements in 1.2, 0.45 and 0.1 µm pore size filtrates. At  
171 the beginning of the anoxic phase (2 hour), potassium nitrate (KNO<sub>3</sub>) was added in order to raise  
172 the initial concentration of NO<sub>3</sub>-N by 20 g N/m<sup>3</sup>. At the beginning of the aerobic phase (6 hour),  
173 the aeration system was turned on and the DO set point was controlled at 6 g O<sub>2</sub>/m<sup>3</sup>.

174  **Figure 2**

175

## 176 **Analytical methods**

177 The samples were sequentially filtered through membrane filters of different pore sizes including  
178 1.2, 0.45 and 0.1 µm pore-size nitrocellulose filters (Billerica MA, USA). The effect of  
179 ultrafiltration was investigated with 0.015 µm pore-size polycarbon filters (Whatman, Kent, UK).

180

181 The TOC and TN concentrations were determined using a TOC analyzer (TOC-V<sub>CSH</sub>) coupled  
182 with a TN module (TNM-1) (SHIMADZU Corporation, Kyoto, Japan). Catalytic thermal  
183 decomposition/chemiluminescence methods, conformed to the American Society for Test  
184 Method's (ASTM) D5176 procedure, are adopted for TN measurement. Samples containing  
185 nitrogen are introduced into an oxygen-rich combustion tube with platinum catalyst at a  
186 temperature of 720 °C. Bound nitrogen is then converted to nitrogen monoxide (NO), further  
187 oxidized to nitrite (NO<sub>2</sub>) in the presence of ozone, and is then detected by a chemiluminescence  
188 detector. TN concentrations in the range of 0.1 to 4000 g/m<sup>3</sup> can be measured.

189

190 The concentrations of inorganic N forms (NH<sub>4</sub>-N, NO<sub>3</sub>-N and NO<sub>2</sub>-N) were determined using





191 Xion 500 spectrophotometer (Dr Lange GmbH, Berlin, Germany). The analytical procedures,  
192 which were adopted by Dr Lange, followed the Standard Methods (APHA, 1992). The  $\text{DON}_{0.1\mu\text{m}}$   
193 and  $\text{DON}_{0.45\mu\text{m}}$  concentrations were calculated as a difference between TN after filtration on the  
194 appropriate pore size filter (i.e. 0.1  $\mu\text{m}$  and 0.45  $\mu\text{m}$ ) and the sum of inorganic N ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$   
195 and  $\text{NO}_2\text{-N}$ ) fractions. The CON concentrations were calculated as a difference between TN after  
196 filtration on the 1.2  $\mu\text{m}$  pore size filter and the sum of  $\text{DON}_{0.1\mu\text{m}}$  and inorganic N fractions.

197

## 198 RESULTS AND DISCUSSION

### 199 ON characteristics in primary and secondary effluent

200 The TON accounted for 24-45% of the TN in primary effluent composite samples from 7 of the  
201 studied WWTPs (excluding the smallest plant, Koscierzyna WWTP) and the average primary  
202 effluent TON concentrations in those plants ranged from 15.5  $\text{g N/m}^3$  (Elblag WWTP) to 35.0  $\text{g}$   
203  $\text{N/m}^3$  (Tczew WWTP) (Table 1). For Koscierzyna WWTP, the average primary effluent TON  
204 concentration and TON/TN ratio were much higher, i.e. 61.0  $\text{g N/m}^3$  and 57%, respectively. The  
205 detailed fractionation of primary and secondary effluent ON (including  $\text{DON}_{0.1\mu\text{m}}$ , low and high  
206 CON and PON) is presented in Figure 3. The primary effluent PON fraction (PON/TON) was  
207 above 50% for all the WWTPs, with the highest at Koscierzyna WWTP (78%), at 60-70% for 3  
208 WWTPs (Gdansk, Gdynia and Tczew), and near 50% for the remaining four WWTPs (Elblag,  
209 Slupsk, Lebork and Kartuzy). For the latter, the colloidal fraction (0.1-1.2  $\mu\text{m}$ ) of the TON was  
210 very significant (36-44%), whereas it ranged from 17 to 34% for the other WWTPs. The average  
211  $\text{DON}_{0.1\mu\text{m}}$  concentrations accounted for only 4-13% of TON and ranged from 1.1  $\text{g N/m}^3$  (Elblag  
212 WWTP) to 3.9  $\text{g N/m}^3$  (Lebork WWTP).

213  **Figure 3**

214

215 The average secondary effluent TN concentrations in the WWTPs ranged from 6.5 to 14.1  $\text{g N/m}^3$ . In  
216 all the plants, the concentrations of  $\text{NH}_4\text{-N}$  were low ( $<1.3 \text{ g N/m}^3$ ) and the dominating form of N



217 was  $\text{NO}_x\text{-N}$  (47-73%). The TON portion constituted 23-35% of the effluent TN, and ranged from  
218  $1.9 \text{ g N/m}^3$  (Tczew WWTP) to  $4.3 \text{ g N/m}^3$  (Koscierzyna WWTP). The average contributions of  
219  $\text{DON}_{0.1\mu\text{m}}$ , CON and PON varied within the ranges of 12-45% ( $0.5\text{-}1.3 \text{ g N/m}^3$ ), 35-44% ( $0.7\text{-}1.9$   
220  $\text{g N/m}^3$ ) and 20-43% ( $0.4\text{-}1.9 \text{ g N/m}^3$ ), respectively. The  $\text{DON}_{0.1\mu\text{m}}$  and CON concentrations were  
221 reduced to a different extent compared to the primary effluent, i.e. on average 56 and 88%,  
222 respectively. As a consequence, the average  $\text{DON}_{0.1\mu\text{m}}/\text{CON}$  ratio increased from 0.24 in the  
223 primary effluent to 0.85 in the secondary effluent. The secondary effluent  $\text{DON}_{0.1\mu\text{m}}$   
224 concentrations were not (or poorly) correlated to any ON form in the primary effluent (the highest  
225 correlation,  $R^2 = 0.29$ , was found with respect to the primary effluent CON concentration). In  
226 contrast, a good correlation ( $R^2 = 0.55$ ) was found between the secondary effluent  $\text{DON}_{0.45\mu\text{m}}$   
227 concentrations and primary effluent CON.

228  
229 Ultrafiltration ( $0.015 \mu\text{m}$ ) after  $0.10 \mu\text{m}$  filtration of the Gdansk and Gdynia WWTP effluents  
230 resulted in similar and relatively small amounts of  $\text{DON}_{0.10\mu\text{m}}$  and dissolved organic carbon  
231 ( $\text{DOC}_{0.10\mu\text{m}}$ ) removal. The average reductions in  $\text{DON}_{0.10\mu\text{m}}$  and  $\text{DOC}_{0.10\mu\text{m}}$  at both plants were 10-  
232 13% and 2-3%, respectively (Table 2). Thus, it can be assumed that the  $0.10 \mu\text{m}$  filtration  
233 provides a reasonable approximation of the DON. The DON consists of both low and high  
234 molecular weight (LMW and HMW) compounds as shown by Pehlivanoglu-Mantas and Sedlak  
235 (2008) for which almost all of a secondary effluent DON passed through a 10 kDa filter ( $0.005$   
236  $\mu\text{m}$ ). About half of the DON passed through a 1 kDa filter and represents LMW substances,  
237 which may include free and combined amino acids and synthetic organics. Nitrogen may also be  
238 present in humic acids at the 3-10 kDa size and higher molecular weight range. In the earliest studies  
239 on the characteristics of DON in secondary effluents, Keller et al. (1978) and Parkin and McCarty  
240 (1981c) reported the percentage of LMW nitrogen compounds ( $<1.8 \text{ kDa}$ ) in the range of 50-66%.  
241 Bratby et al. (2008) also noted a dominance of LMW DON from a previous study, in which 78% of  
242 a secondary effluent DON was at a molecular size of 1.0 kDa or less.



243  **Table 2**

244

245 The average primary and secondary effluent TOC concentrations varied from 125-320 g C/m<sup>3</sup>  
246 and 9.4-17.8 g C/m<sup>3</sup>, respectively (detailed data not shown). Biological treatment consistently  
247 increased the DON/DOC ratio of the wastewater for both conventional (0.45 µm pore size) solids  
248 separation filtration and the 0.1 µm pore size approaching the true dissolved filtrate (Figure 4). In  
249 general, the smaller pore size filtrate had a higher ON/OC ratio suggesting that the colloids in the  
250 pore size range of 0.45-0.1 µm contain proportionally higher amounts of ON than OC. A similar  
251 switch of the DON/DOC ratio was also found by Dignac et al. (2000b) in a conventional activated  
252 sludge pilot plant treating municipal wastewater (SRT = 10 d). The elemental analysis after  
253 electro dialysis (ED) revealed that the ON/OC atomic ratio increased from 0.07 (influent  
254 wastewater) to 0.12 (ozonated sludge) and 0.14 (conventional sludge). Lower degradation rates  
255 for ON would increase the secondary effluent DON/DOC ratio, as observed the full scale  
256 facilities evaluated here, which agrees with Parkin and McCarty (1981a) reporting that the  
257 observed degradation rate of DON<sub>0.45µm</sub> in raw wastewater was less than 50% of the degradation  
258 rate of dissolved COD. The authors attributed this change to the presence of heterocyclic nitrogen  
259 compounds, such as nucleic acid bases, which can yield high N/COD ratios. Westerhoff and Mash  
260 (2002) noted that the DON/DOC ratios are >0.1 in receiving waters affected by wastewater  
261 discharges, agricultural activity or high algae productivity, whereas the DON/DOC ratios are  
262 <0.025 in receiving waters not affected by WWTP effluents or other sources of N pollution.

263  **Figure 4**

264

265 The effluent ON concentrations observed in the northern Poland WWTPs are within the range  
266 reported for municipal WWTPs in the USA Chesapeake Bay region, where very stringent effluent  
267 TN limits of 3 g N/m<sup>3</sup> have been set to help control eutrophication. This study's WWTP data and

268 USA effluent DON data (WERF, 2008; Sattatayewa et al., 2009b) were plotted in Figure 5 to  
269 show the probability distributions of the DON concentrations. WERF (2008) summarized the  
270 reported effluent DON data from 32 BNR facilities across the USA and found that effluent DON  
271 concentrations (without defining the filter pore-sizes used at each plant) varied in the range of 0.1-  
272 2.8 g N/m<sup>3</sup> with the 50 and 90 percentile values of 1.2 and 2.1 g N/m<sup>3</sup>, respectively. The same  
273 figure also presents the distribution of effluent DON data from a recent study (Sattatayewa et al.,  
274 2009b) for 7 US BNR plants. The average DON<sub>0.45µm</sub> concentrations in three samples collected at  
275 each plant ranged from 0.6 to 1.4 g N/m<sup>3</sup> and the 50 and 90 percentile values were 0.9 and 1.7 g  
276 N/m<sup>3</sup>, which is significantly lower in comparison with the results of the 32 plants presented by  
277 WERF (2008). It should be noted that at two WWTPs (Blue Plains, DC and Stamford, CT), also  
278 included in the review of WERF (2008), the DON<sub>0.45µm</sub> concentrations found by Sattatayewa et al.  
279 (2009b) were lower by 0.6 and 0.9 g N/m<sup>3</sup>, respectively. For comparison, very similar values of the  
280 50 and 90 percentiles, i.e. 0.9 and 1.3 g N/m<sup>3</sup>, were obtained for the Polish plants but with respect  
281 to the DON<sub>0.1µm</sub> concentrations.

282  **Figure 5**

283  
284 Similar ranges of ON concentration were also reported for other US WWTPs. In the study of  
285 Pehlivanoglu-Mantas and Sedlak (2008), the DON<sub>0.2µm</sub> concentration (in the samples passed  
286 through 0.2 µm pore-size filters) ranged from 0.7 to 2.1 g N/m<sup>3</sup> in the effluents from three WWTPs  
287 (conventional and N removal activated sludge processes, and a biofilm system). A very similar  
288 range (0.5-2.0 g N/m<sup>3</sup>) was reported by Sattatayewa et al. (2010) in four BNR plants regardless of  
289 the effluent TN concentrations (<5 to 14 g N/m<sup>3</sup>). At another three WWTPs (conventional and N  
290 removal activate sludge processes), Westgate and Park (2010) found the effluent TON  
291 concentrations in the range of 0.9-1.7 g N/m<sup>3</sup> (7-29% of TN) at very low effluent VSS  
292 concentrations (1.3-6.1 g/m<sup>3</sup>).

293



294 The effluent DON consists of compounds which are difficult to remove or produced during  
295 biological treatment (Pehlivanoglu-Mantas and Sedlak, 2008). Despite the variety of ON-  
296 containing compounds detected in municipal WWTP effluents, most of the compounds could not  
297 be identified with available methods. The authors estimated the contributions of some groups of  
298 the compounds as: 1) 10-20% - dissolved free and combined amino acids (these compounds are  
299 most likely produced during biological treatment since amino acids and proteins are readily  
300 biodegradable), 2) <5% ethylenediaminetetraacetic acid (EDTA), and 3) 10% - humic substances  
301 originating from the drinking water sources.

302

303 A relatively low amino acid fraction in municipal WWTP effluent DON of only about 10% was  
304 also noted by Parkin and McCarty (1981c) and Dignac et al. (2000a). Furthermore, Dignac et al.  
305 (2000a) concluded that some of the difficulties in identifying DON with common analytical  
306 methods may be due to the presence of complex structures, which are concentrated during the  
307 biological treatment as a result of the resistance to microbial degradation. Parkin and McCarty  
308 (1981c) suggested that a combination of nucleic acid degradation products, nucleic acid bases,  
309 and heterocyclic nitrogen compounds could account for up to 25% of the effluent  $\text{DON}_{0.45\mu\text{m}}$ .  
310 Westgate and Park (2010) found a strong correlation between protein-N and ON concentrations in  
311 the effluent wastewater. The protein-N constituted a substantial fraction (approximately 60%) of  
312 effluent ON. However, the authors admitted that this value may be overestimated due to two  
313 potential sources of the errors: (1) humic substances that are present in effluents can interfere with  
314 the Lowry protein measurement, (2) inaccuracy of the ON determination by subtracting inorganic  
315 forms from the TN. It should also be noted that the analysis was performed on non-filtered  
316 samples (see above)).

317

### 318 **Fate of DON/CON in activated sludge bioreactors**

319 *Measurement of DON/CON concentration profiles inside bioreactors.* Examples (fall and spring



320 measurement series) of  $\text{DON}_{0.1\mu\text{m}}$  and CON profiles in the full-scale bioreactors at the Gdansk  
321 and Gdynia WWTPs are presented in **Figure 6**. During the measurements, the sum of CON and  
322  $\text{DON}_{0.1\mu\text{m}}$  concentrations in the bioreactor effluent remained in a narrow range, i.e. 1.9-2.3 g N/m<sup>3</sup>  
323 (Gdansk WWTP) and 2.1-2.4 g N/m<sup>3</sup> (Gdynia WWTP). For comparison, the corresponding  
324 average concentrations during the comprehensive survey were 1.7 g N/m<sup>3</sup> (standard deviation  
325 0.32 g N/m<sup>3</sup>) and 2.2 g N/m<sup>3</sup> (standard deviation 0.86 g N/m<sup>3</sup>) at the Gdansk and Gdynia  
326 WWTPs, respectively.

327

328 At the Gdansk plant, the primary effluent  $\text{DON}_{0.1\mu\text{m}}$  concentrations ranged from 1.1 to 2.2 g N/m<sup>3</sup> and  
329 these values are comparable to the results of the comprehensive survey (average concentration 1.8  
330 g N/m<sup>3</sup>, standard deviation 2.42 g N/m<sup>3</sup>). For modeling N conversions in that plant, **Makinia et al.**  
331 **(2011)** assumed that only 10% of the primary effluent DON (<0.2 g N/m<sup>3</sup>) and CON (>1.0 g  
332 N/m<sup>3</sup>) was non-biodegradable, which ultimately accounted for approximately 50% of the sum of  
333 effluent DON and CON concentrations. For comparison, **Parkin and McCarty (1981a)** estimated  
334 that under optimal conditions (SRT = 6-10 d), inert substances from the influent wastewater  
335 represented 60-80% of the effluent  $\text{DON}_{0.45\mu\text{m}}$ , whereas the remaining portion was produced during  
336 activated sludge treatment by the biomass.

337

338 Low concentrations of  $\text{DON}_{0.1\mu\text{m}}$  were observed in all the sampling points along the MUCT  
339 bioreactor. A minor increase in the last (aerobic) compartment was compensated by a reduction of  
340 the “low” (0.1-0.45  $\mu\text{m}$ ) colloidal subfraction. The effluent  $\text{DON}_{0.1\mu\text{m}}$  concentrations ranged from  
341 0.3-1.1 g N/m<sup>3</sup>, which is relatively low when compared to the results of the comprehensive survey  
342 (average concentration 1.2 g N/m<sup>3</sup>, standard deviation 0.71 g N/m<sup>3</sup>). The largest reductions of  
343 both colloidal subfractions were found to occur in the anaerobic and anoxic compartments, which  
344 is substantially affected by influent dilution and by the recirculated mixed liquor. In the anaerobic  
345 compartment, the concentrations of “low” (0.1-0.45  $\mu\text{m}$ ) and “high” (0.45-1.2  $\mu\text{m}$ ) colloidal



346 subfractions decreased by 2.7-3.0 g N/m<sup>3</sup> and 2.1-2.5 g N/m<sup>3</sup>, respectively. In the anoxic  
347 compartment, the corresponding maximum reductions were 0.8-1.0 g N/m<sup>3</sup> and 1.9-2.0 g N/m<sup>3</sup>.

348

349 At the Gdynia plant, relatively high primary effluent DON<sub>0.1µm</sub> concentrations (2.4-3.5 g N/m<sup>3</sup>) were  
350 observed in comparison with the results of the comprehensive survey (average concentration 2.2 g  
351 N/m<sup>3</sup>, standard deviation 1.00 g N/m<sup>3</sup>). The DON<sub>0.1µm</sub> concentrations decreased to 0.6-0.9 and 0.3-  
352 0.7 g N/m<sup>3</sup>, respectively, in the anaerobic and anoxic compartment. However, similar to the Gdansk  
353 WWTP, an increase in DON<sub>0.1µm</sub> concentration and decrease in the “low” CON subfraction were  
354 observed under aerobic conditions in the last compartment. The highest reductions of the “low” and  
355 “high” CON subfractions were observed in the anaerobic compartment (by 3.2-4.3 g N/m<sup>3</sup>) and  
356 anoxic compartment (by 1.7-2.4 g N/m<sup>3</sup>), respectively.

357

358 With regard to colloidal and dissolved OC, the dominant primary effluent fraction (75-80%) at  
359 both plants was DOC, which is very similar to the results of the comprehensive survey (see above).  
360 The DOC concentrations primarily decreased in the anaerobic compartment (by 50-70%), whereas  
361 the DOC reduction in the entire bioreactors reached 75-85%. The colloidal fraction was reduced  
362 in the anaerobic compartment and then stabilized. After that, no significant transformations  
363 between the two colloidal subfractions (“low” and “high”) were observed.

364  **Figure 6**

365

366 **Sattayatewa et al. (2009a)** carried out similar measurements of ON concentration profiles in a  
367 full-scale 4-stage Bardenpho bioreactor. The primary effluent DON<sub>0.45µm</sub> concentrations were low,  
368 ranging from 0.84 to 1.37 g N/m<sup>3</sup> with the average value 1.11 g N/m<sup>3</sup>. The results showed ON  
369 release in the primary anoxic compartment and no ON release in the first aerobic compartment of  
370 the activated sludge process. With regard to the DON concentrations determined in the samples  
371 after pretreatment using the coagulation-flocculation method of **Mamais et al. (1993)** (DON<sub>ff</sub>), a



372 decreasing trend was observed along the treatment train until the first anoxic compartment and  
373 then remained stable in the last three compartments (first aerobic/second anoxic/second aerobic).  
374 In the bioreactor effluent,  $\text{DON}_{0.45\mu\text{m}}$  and  $\text{DON}_{\text{ff}}$  were essentially identical. In the follow-up study  
375 (Sattayatewa et al., 2010), however, the effluent  $\text{DON}_{\text{ff}}/\text{DON}_{0.45\mu\text{m}}$  in four BNR bioreactors  
376 ranged from 0.76 to 0.95 suggesting a significant contribution of the “low” CON subfraction to  
377 the effluent ON.

378

379 ***Determination of DON/CON behavior in bench-scale experiments.*** The average CON and  
380  $\text{DON}_{0.1\mu\text{m}}$  concentrations ( $\pm$ standard deviations) for all the experiments are summarized in Table  
381 3, and Figure 7 illustrates an example of the behavior of CON and  $\text{DON}_{0.1\mu\text{m}}$  profiles measured at  
382 the Gdansk and Gdynia WWTPs plants during the summer testing. In all the cases,  $\text{DON}_{0.1\mu\text{m}}$  was  
383 explicitly produced in the aerobic compartment of the anaerobic/anoxic/aerobic process. For the  
384 Gdansk batch tests, the  $\text{DON}_{0.1\mu\text{m}}$  concentration increases ranged from 0.6-1.9 (average 0.9) g  
385  $\text{N}/\text{m}^3$  when fed pre-settled wastewater influent (reactor 1) and ranged from 0.2-1.3 (average 0.7) g  
386  $\text{N}/\text{m}^3$  when fed coagulated-flocculated wastewater influent (reactor 2). Corresponding results for  
387 the Gdynia WWTP were concentration increases of 0.3-1.1 (average 0.5) g  $\text{N}/\text{m}^3$  (reactor 1) and  
388 0.4-1.6 (average 0.8) g  $\text{N}/\text{m}^3$  (reactor 2), respectively. These findings are in accordance with the  
389 observations in the full-scale activated sludge process in which  $\text{DON}_{0.1\mu\text{m}}$  concentrations also  
390 increased in the aerobic compartment. Furthermore, in order to evaluate if the increase under  
391 aerobic conditions was statistically significant, unpaired t-tests with unequal variances were  
392 performed on two data sets ( $\text{DON}_{0.1\mu\text{m}}$  concentrations at the end of anoxic and aerobic  
393 period/zone). The calculated p-value, which is the probability of observing equal concentrations  
394 for these sampling points, varied at both studied plants in the range of 0.008-0.009 and 0.05-0.09,  
395 respectively, for the batch tests and full-scale measurements. Such low p-values, especially for the  
396 batch tests, suggest that the examined increase was statistically significant.

397






398 It should also be noted that the behavior of inorganic N forms ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ) was  
399 similar in the batch tests with the process mixed liquor from both WWTPs (Makinia et al., 2009).  
400 However, complete nitrification could not be achieved due to high initial  $\text{NH}_4\text{-N}$  concentrations  
401 ranging from 35 to 40  $\text{g N/m}^3$ , and  $\text{NO}_2\text{-N}$  accumulated to a peak concentration of approximately 9  
402  $\text{g N/m}^3$  in one test at the Gdansk WWTP. During the other tests, the peak  $\text{NO}_2\text{-N}$  concentrations  
403 ranged from 2 to 4  $\text{g N/m}^3$ . In contrast, no  $\text{NO}_2\text{-N}$  accumulation was observed during the  
404 measurements either in the aerobic compartments of the full-scale bioreactors or in the secondary  
405 effluents during routine samplings by the plant operators.

406

407 In contrast to the full-scale measurements, the CON profile behaviors in the anaerobic and anoxic  
408 phases were different for the two WWTPs. During the Gdansk WWTP batch experiment, an  
409 apparent production of DON from CON hydrolysis occurred in the anoxic phase. This finding  
410 was derived based on the observation that lower amounts of CON were utilized and higher  
411 amounts of DON were produced in reactor 2 (containing exclusively the soluble fraction)  
412 compared to reactor 1 (containing all the fractions). These results are consistent with the full-scale  
413 measurements at that plant. On the other hand, the batch test CON behavior was the opposite in  
414 the Gdynia batch tests, with only a slight decrease in CON (reactor 1) or increase (reactor 2)  
415 (Table 3).

416  **Table 3**

417  **Figure 7**

418

419 Until now, the only laboratory experiments evaluating the behavior of ON in activated sludge  
420 were reported by Parkin and McCarty (1981b,c). Parkin and McCarty (1981c) carried out a series  
421 of eight aeration batch tests (up to 72 h) and the average ultimate  $\text{DON}_{0.45\mu\text{m}}$  concentration was  
422 1.4 ( $\pm 0.46$ )  $\text{g N/m}^3$ . Based on the N mass balance, the authors estimated that 52% of it was

423 recalcitrant (resistant to biological transformations) from the influent wastewater sources, 19%  
424 was produced from biomass endogenous decay in the activated sludge process (this portion is a  
425 function of the biomass concentration and SRT, 16% was in equilibrium between that sorbed to  
426 biomass and that in the liquid (this portion is independent of the biomass concentration and  
427 temperature, but dependent on cultural characteristics) and about 13% could be further degraded.  
428 Furthermore, the authors noted that increasing the activated sludge SRT could either degrade  
429 further influent  $\text{DON}_{0.45\mu\text{m}}$  or it could also raise  $\text{DON}_{0.45\mu\text{m}}$  via biomass endogenous respiration.  
430 The compounds released during biomass decay were defined as “poorly biodegradable”.  
431 Furthermore, **Parkin and McCarty (1981b)** observed peak  $\text{DON}_{0.45\mu\text{m}}$  concentrations (0.1 to 0.6 g  
432  $\text{N}/\text{m}^3$ ) during the initial 6 hours of aeration batch tests regardless of initial substrate, MLSS and  
433  $\text{NH}_4\text{-N}$  concentrations, and a substrate type. The authors attributed that excretion of DON to  
434 concentration gradients, starvation conditions, addition of exogenous substrate, and changes in  
435 phase and rate of growth. Excreted  $\text{DON}_{0.45\mu\text{m}}$  was subsequently utilized by the microorganisms,  
436 leading to a minimum  $\text{DON}_{0.45\mu\text{m}}$  concentration after 4 to 8 hours. Starvation conditions then  
437 prevailed, and a gradual increase in  $\text{DON}_{0.45\mu\text{m}}$  concentration occurred due to microorganism  
438 decay. This finding is consistent with the observations of the bench-scale experiments at the  
439 Gdansk and Gdynia WWTPs where minimum  $\text{DON}_{0.1\mu\text{m}}$  concentrations were observed at the end  
440 of the anoxic phase (after 6 hours from the beginning of the experiment).

441

442

## 443 CONCLUSIONS

444 From this study in the 8 BNR WWTPs, the following conclusions can be derived:

- 445 ■ The average primary effluent  $\text{DON}_{0.1\mu\text{m}}$  concentrations ranged from 1.1  $\text{g N}/\text{m}^3$  to 3.9  $\text{g N}/\text{m}^3$   
446 and  $\text{DON}_{0.1\mu\text{m}}$  accounted for only 4-13% of TON concentrations which varied in a wide range  
447 from 15.5 to 61  $\text{g N}/\text{m}^3$ . The fraction of  $\text{DON}_{0.1\mu\text{m}}$  increased to 12-45% of the effluent TON

448 following BNR activated sludge treatment, but the average secondary effluent  $\text{DON}_{0.1\mu\text{m}}$   
449 concentrations ranged from only 0.5 to 1.3 g N/m<sup>3</sup> which implies the biodegradability of a  
450 major portion of the primary effluent  $\text{DON}_{0.1\mu\text{m}}$ .

451 ■ The relatively narrow range of secondary effluent  $\text{DON}_{0.1\mu\text{m}}$  concentrations was observed  
452 despite high variations in the size of the studied plants (37,000-565,000 PE), biological  
453 process configuration employed (UCT, MUCT, JHB, A<sub>2</sub>/O, MLE) and operating parameters  
454 (SRT = 9-34 d). Furthermore, the secondary effluent  $\text{DON}_{0.1\mu\text{m}}$  concentrations were not (or  
455 poorly) correlated to any ON form in the primary effluent (the highest correlation,  $R^2 = 0.29$ ,  
456 was found with respect to the primary effluent CON concentration). In contrast, a good  
457 correlation ( $R^2 = 0.55$ ) was found between the secondary effluent  $\text{DON}_{0.45\mu\text{m}}$  concentrations  
458 and primary effluent CON.

459 ■ The secondary effluent CON fraction accounted for 43 to 78% of the non-particulate ON (<1.2  
460  $\mu\text{m}$ ) with the remainder as  $\text{DON}_{0.1\mu\text{m}}$ . Ultrafiltration with 0.015  $\mu\text{m}$  pore size filters had only a  
461 minor effect on further reductions of the effluent  $\text{DON}_{0.1\mu\text{m}}$ .

462 ■ The largest reductions of the CON fractions were found to occur in the anaerobic and anoxic  
463 compartments of the studied bioreactors, whereas an increase of  $\text{DON}_{0.1\mu\text{m}}$  concentrations was  
464 observed in the aerobic compartment. During batch experiments with process mixed liquor and  
465 primary effluent wastewater,  $\text{DON}_{0.1\mu\text{m}}$  was consistently produced in the aerobic phase.

466

467

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475

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553 **Table 1. Basic characteristics of the studied BNR WWTPs**

554 **Table 2. DON and DOC before and after ultrafiltration of the secondary effluents at the**

555 **Gdansk and Gdynia WWTPs**

556 **Table 3. Average CON and DON concentrations during the 3-phase**

557 **anaerobic/anoxic/aerobic batch experiments at the Gdansk and Gdynia WWTPs**

558





559 **Table 1. BNR WWTPs configuration, average flowrate, SRTs, and plant influent and**  
 560 **effluent TN concentrations for 2007-2008 (based on the routine operating data)**  
 561  
 562

Facility	Size	Average flowrate	SRT	Bioreactor configuration	Average TN (2007-08)	
					Influent	Effluent
	PE	m <sup>3</sup> /d	d		gN/m <sup>3</sup>	gN/m <sup>3</sup>
Gdansk	565,000	81,000	21-31	MUCT	81.2	11.6
Gdynia	516,000	56,000	14-27	JHB	82.5	12.9
Elblag	181,000	36,000	15-22	MLE	66.9	5.6
Slupsk	180,000	19,000	18-29	UCT	73.9	8.4
Tczew	70,000	8,900	19-34	A <sub>2</sub> /O	84.5	6.8
Lebork	56,000	7,200	18-25	JHB	80.7	10.9
Kartuzy	47,000	3,200	9-24	UCT	97.9	14.0
Koscierzyna	37,000	3,200	12-29	JHB	113.0	10.3

563

564

565 **Table 2. DON and DOC before and after 0.10  $\mu\text{m}$  filtration and ultrafiltration of the**  
 566 **secondary effluents at the Gdansk and Gdynia WWTPs**  
 567  
 568

<b>Gdansk WWTP</b>					
Sample	Unit	Test 1	Test 2	Test 3	Average
DON (<0.1 $\mu\text{m}$ )	g N/m <sup>3</sup>	1.88	2.10	1.99	<b>1.99</b>
DON <sub>UF</sub> (<0.015 $\mu\text{m}$ )	g N/m <sup>3</sup>	1.77	2.04	1.59	<b>1.80</b>
DOC (<0.1 $\mu\text{m}$ )	g C/m <sup>3</sup>	17.0	17.5	10.2	<b>14.9</b>
DOC <sub>UF</sub> (<0.015 $\mu\text{m}$ )	g C/m <sup>3</sup>	16.6	17.2	9.4	<b>14.4</b>
<b>Gdynia WWTP</b>					
Sample	Unit	Test 1	Test 2	Test 3	Average
DON (<0.1 $\mu\text{m}$ )	g N/m <sup>3</sup>	1.52	1.63	1.05	<b>1.40</b>
DON <sub>UF</sub> (<0.015 $\mu\text{m}$ )	g N/m <sup>3</sup>	1.26	1.39	1.00	<b>1.22</b>
DOC (<0.1 $\mu\text{m}$ )	g C/m <sup>3</sup>	16.0	10.7	9.9	<b>12.2</b>
DOC <sub>UF</sub> (<0.015 $\mu\text{m}$ )	g C/m <sup>3</sup>	15.8	10.4	9.6	<b>11.9</b>

569

570



571 **Table 3. Average CON and DON concentrations during the 3-phase**  
 572 **anaerobic/anoxic/aerobic batch experiments at the Gdansk and Gdynia WWTPs**  
 573  
 574

Gdansk (average ± standard deviations from 5 experiments) (T = 12.8-16.8 °C, MLVSS = 1.17-3.04 kg/m <sup>3</sup> )						
	Reactor 1 (without pretreatment)			Reactor 2 (with pretreatment)		
Sample	DON	CON	Total	DON	CON	Total
Start	0.6 ± 0.21	3.3 ± 1.34	3.9 ± 1.53	0.6 ± 0.56	2.1 ± 0.84	2.7 ± 1.20
Anaerobic	0.8 ± 0.52	3.3 ± 1.04	4.1 ± 1.43	0.8 ± 0.42	2.1 ± 0.84	2.9 ± 1.15
Anoxic	0.7 ± 0.33	2 ± 0.33	2.7 ± 0.50	0.2 ± 0.08	2.1 ± 0.88	2.3 ± 0.91
Aerobic	1.6 ± 0.55	2.1 ± 0.76	3.7 ± 0.38	0.9 ± 0.42	1.7 ± 1.00	2.6 ± 0.82

Gdynia – (average ± standard deviations from 6 experiments) (T = 13.1-17.9 °C, MLVSS = 1.21-2.57 kg/m <sup>3</sup> )						
	Reactor 1 (without pretreatment)			Reactor 2 (with pretreatment)		
Sample	DON	CON	Total	DON	CON	Total
Start	0.8 ± 0.38	2.4 ± 0.84	3.2 ± 1.00	0.7 ± 0.32	1.8 ± 0.93	2.5 ± 0.78
Anaerobic	0.8 ± 0.45	2.1 ± 0.77	2.9 ± 1.03	0.9 ± 0.51	1.8 ± 0.56	2.7 ± 0.90
Anoxic	0.6 ± 0.28	1.9 ± 0.8	2.5 ± 1.03	0.6 ± 0.17	2.3 ± 1.17	2.9 ± 1.12
Aerobic	1.1 ± 0.58	2.2 ± 0.62	3.3 ± 1.12	1.4 ± 0.70	2.4 ± 1.18	3.8 ± 1.38

575  
 576

577 **LIST OF FIGURES**

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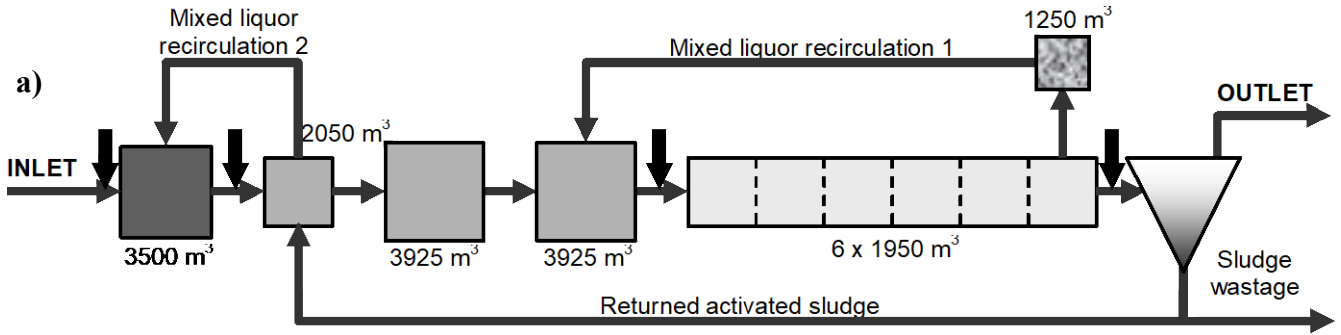
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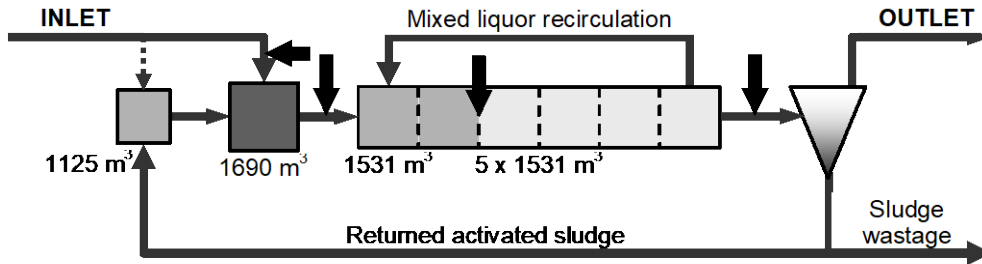


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anaerobic zone
  anoxic zone
  aerobic zone
  deoxic zone

b)



anaerobic zone
  anoxic zone
  aerobic zone

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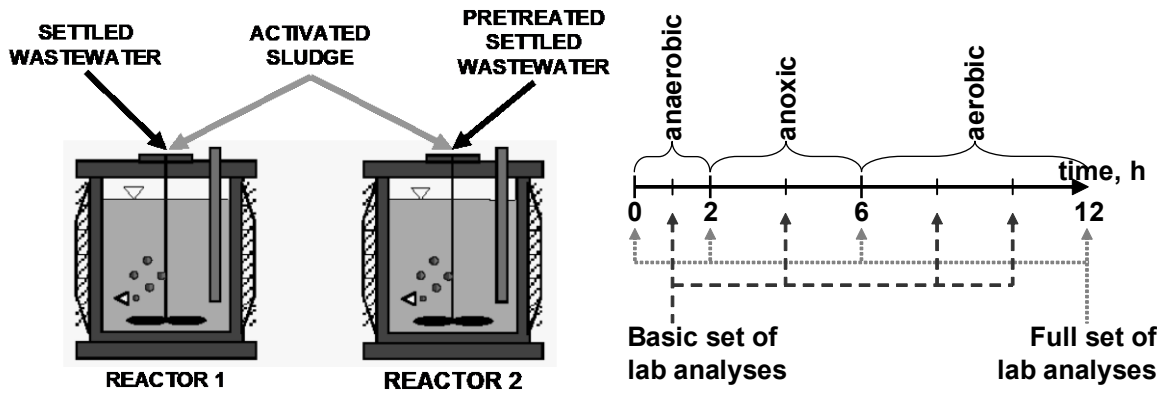
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**Figure 1. Schematic of BNR system bioreactor compartments: (a) Gdansk MUCT process, (b) Gdynia JHB process (black arrows show sampling locations)**

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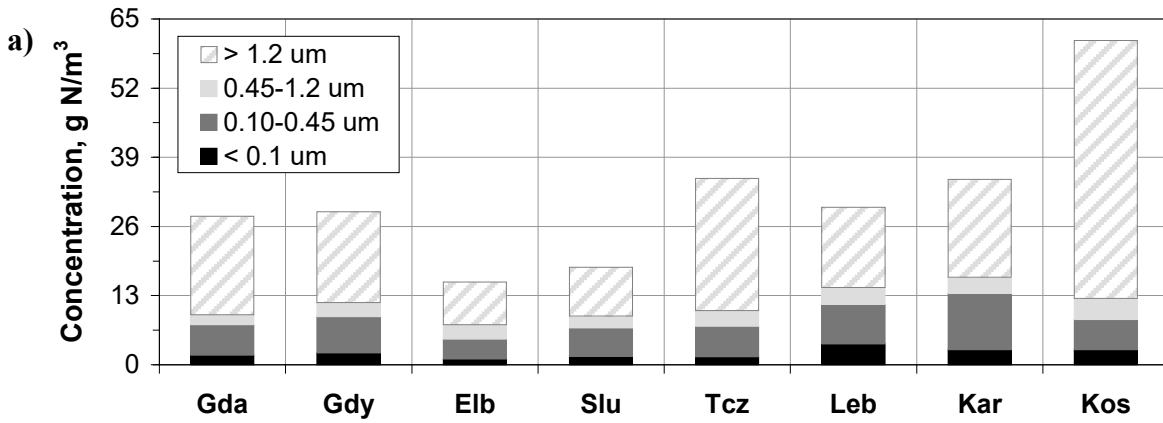
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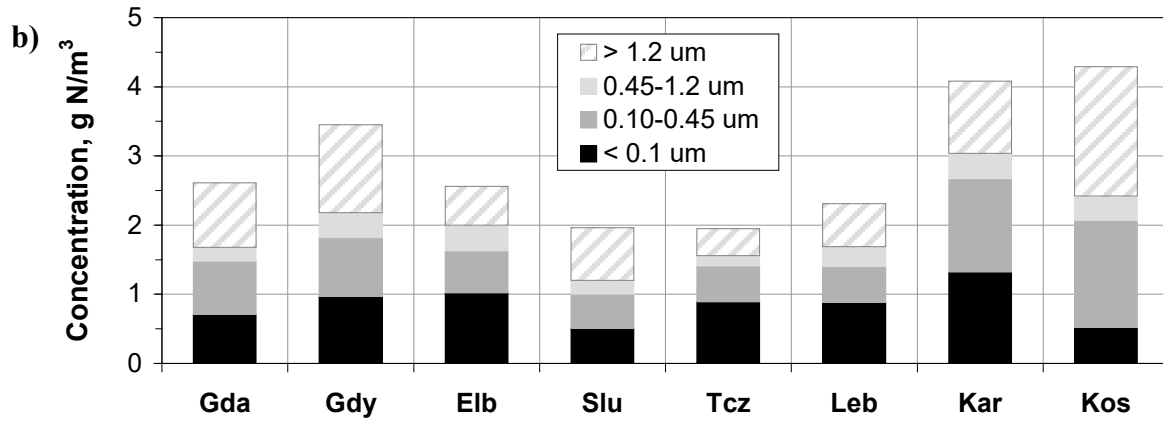
613 **Figure 2. Feed source, operating sequence, and analyses for experiments with two**  
614 **parallel batch reactors (parameters measured in the basic and full sets are described in**  
615 **the text)**

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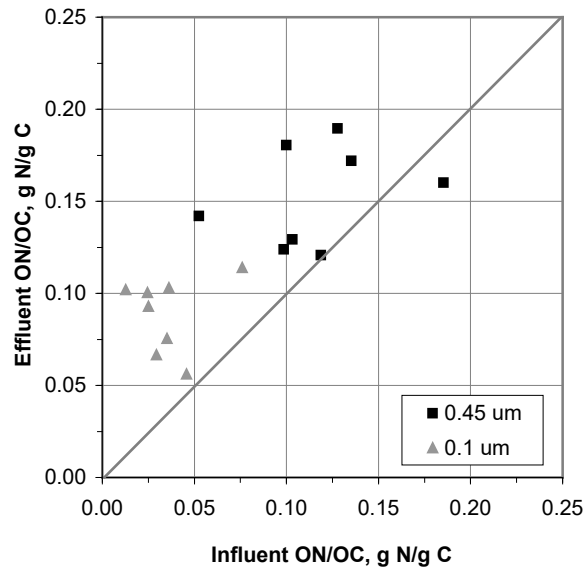
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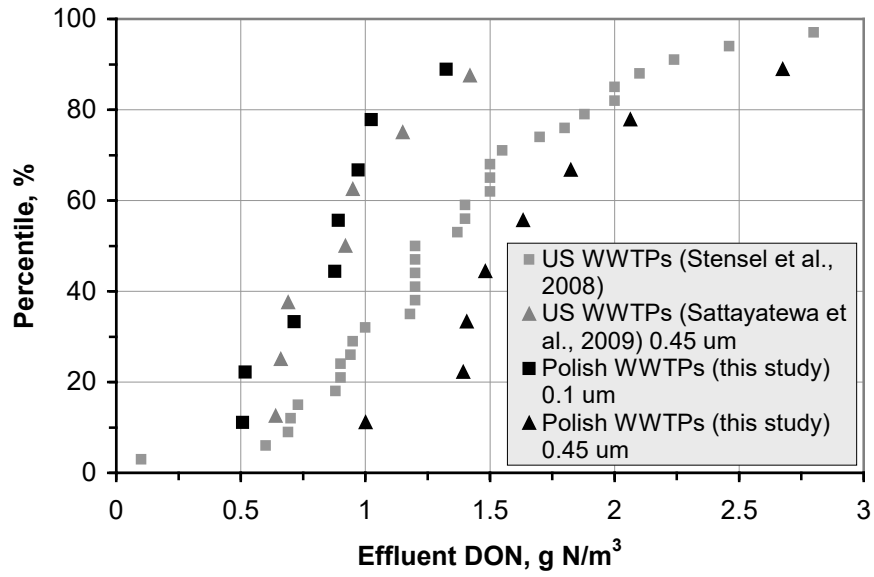
Figure 3. Primary and secondary effluent ON fractions in the 8 studied BNR WWTPs



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**Figure 4. Effluent DON/DOC vs. influent DON/DOC in the 8 studied BNR WWTPs based on filtration through 0.45 µm and 0.1 µm pore-size filters**

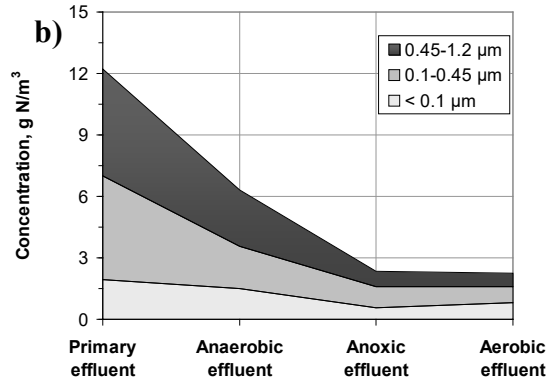
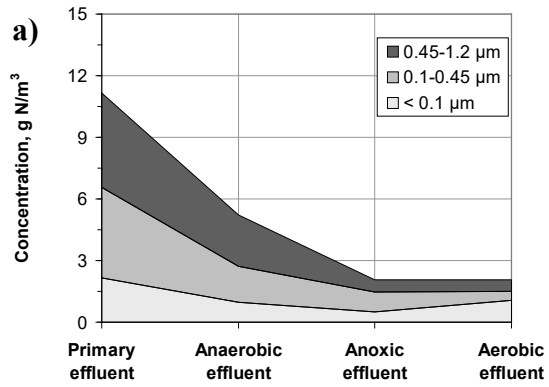




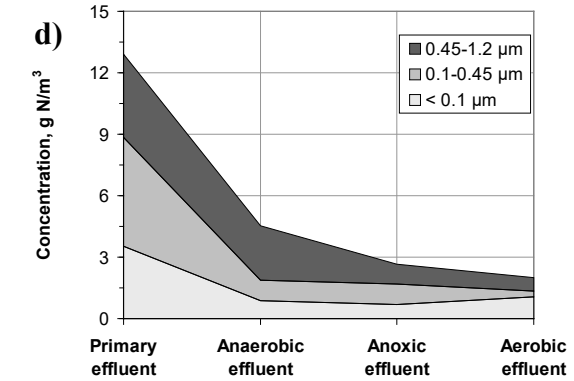
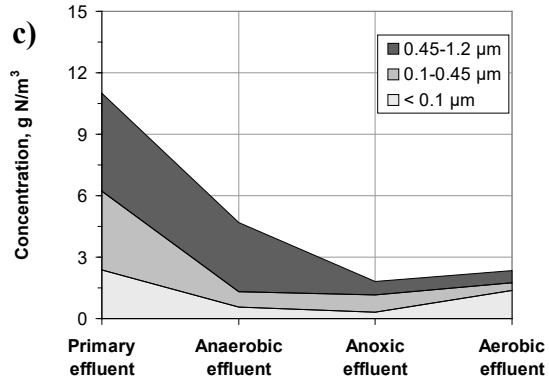
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**Figure 5. Probability distributions of secondary effluent DON concentrations**

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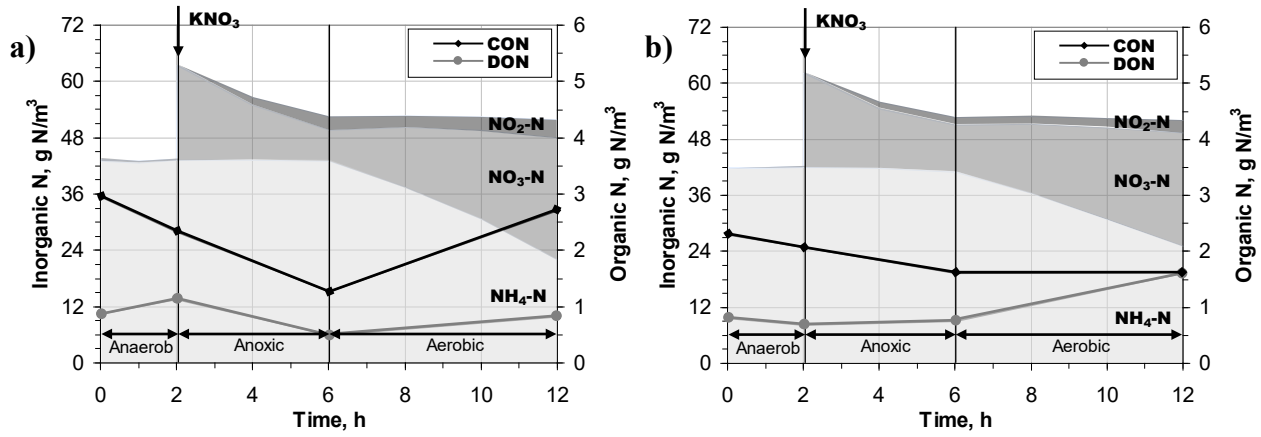
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652 **(fall and spring study sessions) at the Gdansk WWTP (a-b) and the Gdynia WWTP (c-d)**

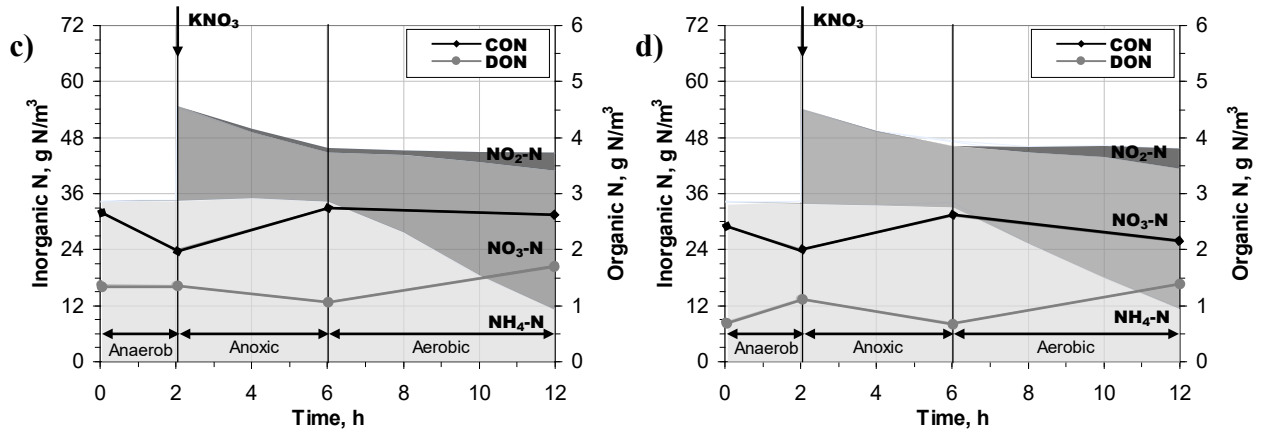
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**Figure 7. Behavior of nitrogen compounds during 3-phase batch tests with the full-scale process biomass from the Gdansk WWTP: (a) settled wastewater, (b) settled wastewater after coagulation-flocculation, and the Gdynia WWTP: (c) settled wastewater, (d) settled wastewater after coagulation-flocculation**